

Note

Permanent "on column" modification of a silica stationary phase with 1,4-dioxane

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In supercritical fluid chromatography (SFC) with packed columns, it has frequently been observed that adding small amounts of a polar modifier to a non-polar or weakly polar mobile phase yielded considerable effects. Not only retention times decreased more than could be expected from the variation of the solvent strength alone, but also peak shapes were drastically improved^{1–5}. These effects have been ascribed to a modification of the stationary phase in that highly active sites are occupied by the modifier and thus, the activity of the remaining sites becomes more uniform. This hypothesis has been supported by the failure to produce such a modifier effect with low modifier concentration on columns packed with polystyrene¹ and on polysiloxane coated capillary columns⁶.

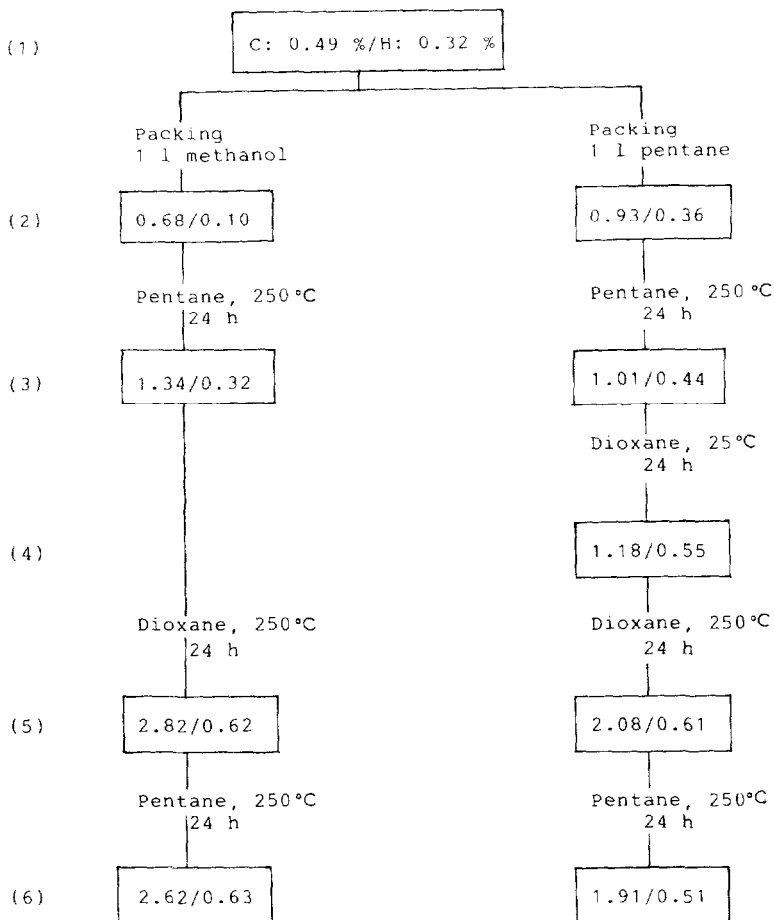
In this communication, we report results on a permanent modification of a silica stationary phase by 1,4-dioxane which supports the theory of the modifier-stationary phase interaction.

EXPERIMENTAL

Instrumentation^{7,8} and mobile phase pretreatment^{9,10} have been described previously.

The stainless-steel separation columns (25 cm × 4.6 mm I.D.) were packed with LiChrosorb Si 100, 10 μm (Merck, Darmstadt, F.R.G.) using a slurry method⁹. The slurry consisted of a suspension of the stationary phase (2.5 g) in a mixture of toluene (20 ml) and cyclohexanol (30 ml). From a reservoir at the top of the column the slurry was packed by pumping methanol or pentane (the packing medium) onto the reservoir. Modification of the stationary phase was performed by passing 1,4-dioxane through the column at a temperature of 250°C, the flow-rate being 1 ml/min (*cf.*, Scheme 1). The consecutive flushing of the columns was done at the same flow-rate with pentane or dioxane. From the columns, stationary phase samples were taken at each step for elemental analysis. These silica samples were thoroughly dried *in vacuo* to constant weight before being analyzed.

For the determination of the column performance, a test mixture of the four aromatic hydrocarbons naphthalene, anthracene, pyrene, and chrysene, dissolved in heptane, was used. The determination of capacity ratios¹¹ and resolution¹² was performed as described previously. A UV detector was used at a wavelength of 254 nm.



Scheme 1. Modification of the silica stationary phase.

RESULTS

During studies of the chromatographic performance of mixed mobile phases containing 1,4-dioxane it was observed that different capacity ratios were obtained depending on the pretreatment of the columns. With freshly packed columns after having removed the packing medium by thoroughly flushing the column with the mobile phase under investigation, highly reproducible separations were obtained with one-component eluents such as pure pentane or pure carbon dioxide. However, when supercritical mixtures containing dioxane were applied, reproducible chromatograms were obtained only after a longer period of use. Furthermore, such dioxane-treated columns showed different characteristics compared to freshly packed columns when pure carbon dioxide or pentane was subsequently used as the mobile phase. As Fig. 1 shows, retention times are smaller and peak shapes are greatly improved with the dioxane-treated silica, even at conditions of very slow elution. Thus, it could be

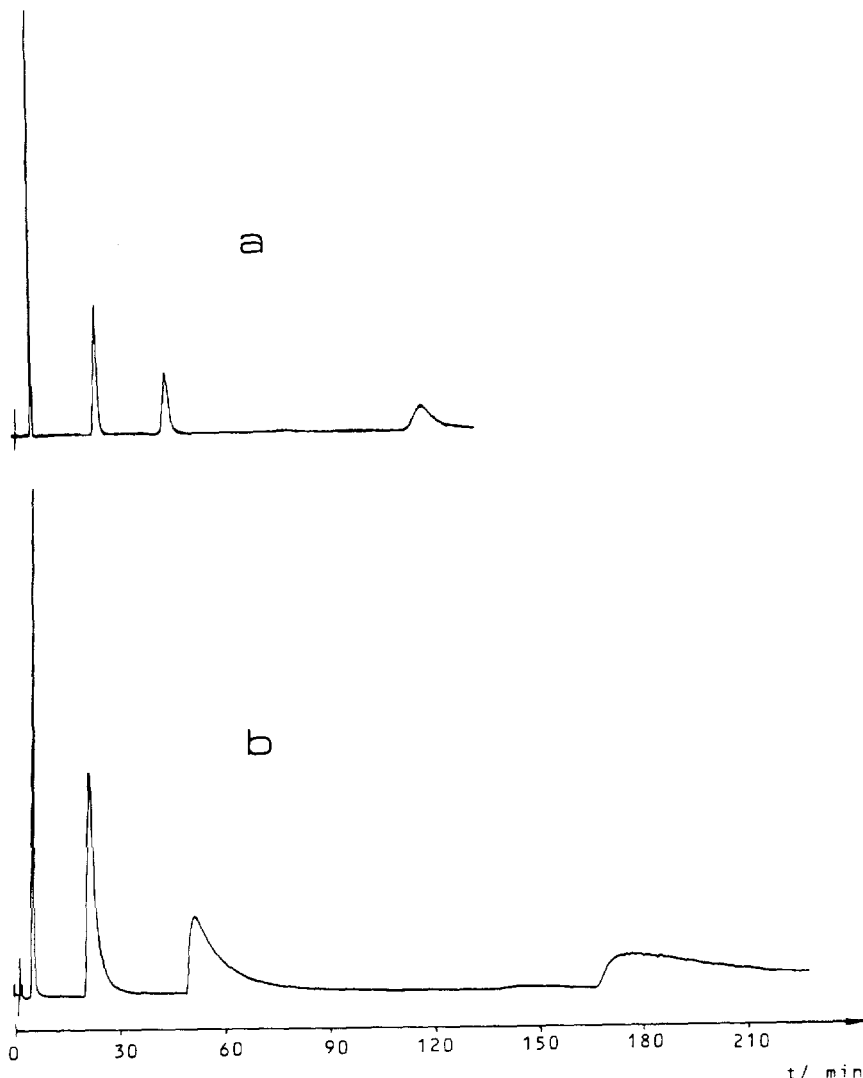


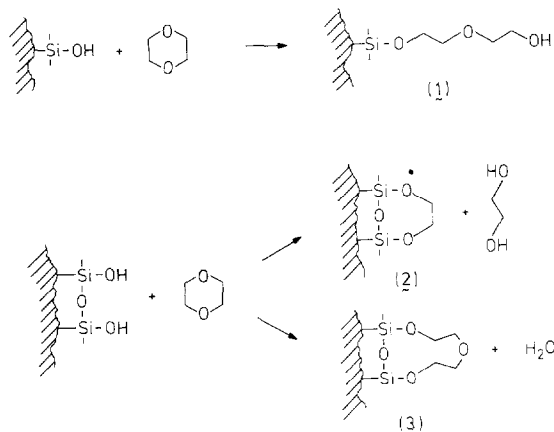
Fig. 1. SFC separations of the aromatic test mixture on modified (a) and unmodified (b) silica. Mobile phase, carbon dioxide; temperature, 120°C; pressure, 150 bar. Column: LiChrosorb Si 100, 10 μm ; column dimensions, 25 cm \times 4.6 mm I.D.

suspected that a permanent modification of the silica stationary phase had occurred.

In view of the finding of Hirata³ that with supercritical hexane-ethanol mixtures an irreversible modification of a silica stationary phase took place, and considering the assumption of this author that this modification might be due to the formation of ethyl-silyl ether groups, we decided to study these phenomena with dioxane treated silica stationary phase in more detail.

Cyclic ethers are known to be cleavable in an acidic environment, and under appropriate conditions they undergo a polymerization reaction. One can postulate

that highly active sites on the silica surface may be able to cleave dioxane molecules, especially at elevated temperatures. This may lead to the formation of ether structures 1–3 on the silica surface, provided that a polymerization reaction does not occur. Thus, the highly active adsorption sites of the stationary phase would be deactivated, and the resulting stationary phase may also show a more uniform activity. Possibilities for the cleavage reaction would be:



The formation of structures of types 1, 2 or 3 introduces carbon atoms into the stationary phase, which can be determined by elemental analysis. Therefore, samples were prepared as described and analyzed, the results being summarized in Scheme 1. In fact, considerable amounts of carbon and hydrogen are found when the silica has been in contact with dioxane at higher temperatures.

The original untreated silica already contained small amounts of carbon and hydrogen [step (1) in Scheme 1]. When the silica had been packed into the columns using methanol or pentane as the packing medium, the amounts of carbon and hy-

TABLE I

PLATE NUMBERS FOR SEVERAL AROMATIC COMPOUNDS ON UNMODIFIED AND DIOXANE-MODIFIED SILICA AT DIFFERENT TEMPERATURES

Eluent, carbon dioxide; pressure, 150 bar; column: LiChrosorb Si 100, 10 μm ; column dimensions, 25 cm \times 4.6 mm I.D.

	T ($^{\circ}\text{C}$)	Plate number (N)			
		<i>Naphthalene</i>	<i>Anthracene</i>	<i>Pyrene</i>	<i>Chrysene</i>
Unmodified silica	20	955	275	105	30
	80	335	430	140	110
	120	585	560	190	75
Dioxane-modified silica	20	1145	1315	1290	1045
	80	930	1745	3600	1590
	120	4265	8805	9205	6450

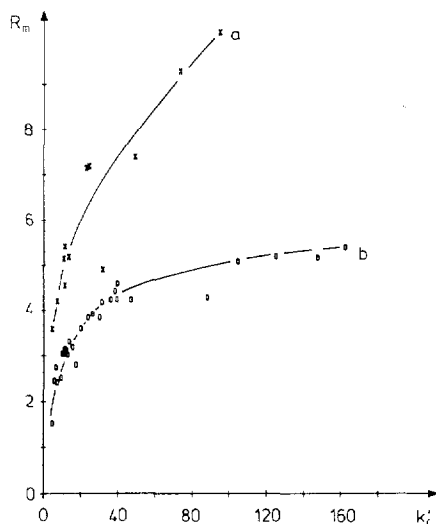


Fig. 2. Plots of average resolution, R_m , of the aromatic test mixture versus chrysene capacity ratio, k'_c , for modified (a) and unmodified (b) silica. Mobile phase, carbon dioxide. Different temperatures and pressures in the range of 40–250°C and 120–250 bar, respectively, have been employed.

drogen increased to some extent (2). After flushing the packed columns with supercritical pentane for 24 h, about 1% carbon was found for both columns (3). At this stage, the behavior of the stationary phase is stable towards carbon dioxide and pentane but can be altered with dioxane-containing mobile phases at elevated (particularly supercritical) temperatures. However, flushing the stationary phase with dioxane at ambient temperature neither changes the carbon content considerably, nor alters the chromatographic properties (4). After flushing with dioxane at 250°C and 70 bar for 24 h, the carbon content reaches 2–3% (5), and the chromatographic behaviour of the columns has changed greatly. Both the carbon content and the chromatographic properties remain constant again upon flushing with supercritical pentane (6).

The changes of the chromatographic properties have been studied using a substrate test mixture of the aromatics naphthalene, anthracene, pyrene, and chrysene. It was found that both capacity ratios and peak widths were considerably reduced after column modification. This is made obvious both from Table I and Fig. 2. Compared to the unmodified silica, the dioxane-treated silica yields considerably higher plate numbers for all aromatic substrates, the improvement being almost two orders of magnitude for chrysene at 120°C. Fig. 2 shows a plot of the average resolution, R_m , for the four aromatics versus the capacity ratio of chrysene, using supercritical carbon dioxide at different pressures and temperatures (40–250°C and 120–250 bar) as the mobile phase. Comparing the curves for the modified (a) and the unmodified (b) silica, resolution is considerably higher with the modified material at equal k' .

CONCLUSIONS

Upon treating a silica stationary phase with 1,4-dioxane at elevated temperature, a modification of the silica surface occurs. This is evidenced by considerable amounts of carbon and hydrogen in the stationary phase as determined by elemental analysis, and by permanent changes of the chromatographic properties of the silica. Whether the modification is due to the formation of silyl ether linkages after ring-cleavage of dioxane molecules or to a ring-opening polymerization reaction, cannot yet be answered. The blocking of highly active sites during the modification leads to improved chromatographic performance of the stationary phase. This applies especially to carbon dioxide as the mobile phase, which yields rather poorly shaped peaks with unmodified silica. However, the general polar nature of the silica appears unchanged. Thus, this modification can be used to produce polar silica stationary phases yielding lower capacity ratios and higher plate numbers and resolution.

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